University of Dundee

MASTER OF SCIENCE

Prevention of cancer therapy related cardiotoxicity
A Tayside Pharmaco-Epidemiology study (PROTECT-TAYSIDE study)

Soujeri, Bayan

Award date:
2018

Prevention of cancer therapy related cardiotoxicity: A Tayside Pharmaco-Epidemiology study (PROTECT-TAYSIDE study)

Bayan Soujeri

MSc by research Medicine

Supervisor: Professor Chim C Lang
Co-Supervisors: Professor Allan D Struthers
Dr Michelle Ferguson

University of Dundee

April 2017

Table of Contents

Illustrations list

Acknowledgements

First and Foremost, I would like to thank my supervisor Professor Chim Lang, for giving me the chance to be part of his excellent research team and the support to make it this far. His guidance, availability and expertise has been invaluable. I would also like to give a special acknowledgement to Dr Michelle Ferguson for overseeing my progress and her input in clarifying matters from an oncological prospective. I would also like to sincerely thank the following people: Dr Jagdeep Singh for his novel ideas and mentoring me throughout the project. Mr Mohapradeep Mohan for his help with getting the project off the ground; his guidance on getting the necessary approvals was crucial in the early stages. first class; Mr Daniel Levin for his excellent help with statistical analysis. I also gratefully acknowledge the help of Ninewells Ward 32 staff Angela Philips and Ann Mckenna. Finally, I would like to say a very special thanks to my good friend Mr Sean Hawkey. Sean offered a medical student prospective and his effort in taking time to collect data for the study was extremely helpful.

Declaration

The candidate is the author of this thesis. The author declares that the content of this project report is his own work and has not previously been submitted for any other assessment. The report is written in the authors own words and conforms to the University of Dundee's Policy on plagiarism and academic dishonesty. Unless otherwise indicated, all of the references cited in this report have been consulted by the author.

I declare that I am the supervisor of the candidate named above and that the conditions of relevant Ordinance and Regulations have been fulfilled.

Abbreviations List:

Abbreviation	Definition
β-Blockers	Beta Blockers
ACEi	Angiotensin Converting Enzyme Inhibitors
AMPK	Adenosine Monophosphate-activated Protein Kinase
ANP	Atrial natriuretic peptide
ANT	Anthracycline
ARBs	Angiotensin receptor blockers
BC	Breast Cancer
BNP	Brain natriuretic peptide
CAD	Coronary Artery Disease
CCBs	Calcium Channel Blockers
CHF	Congestive Heart Failure

CI	Confidence Interval
DOX	Doxorubicin
EDP	End Diastolic Pressure
ESP	End Systolic Pressure
HDC	High Dose Chemotherapy
HF	Heart Failure
IGF-1	Insulin-like Growth Factor-1
IHD	Ischaemic Heart Disease
LDH	Lactate dehydrogenase
LVEF	Left Ventricular Ejection Fraction
LV Mass	Left Ventricular Mass
MUGA	Multigated Radionuclide Angiography
OPS	Oncoplastic Surgery
SERM	Selective estrogen receptor modulator
TOP 2	Topoisomerase II
TZDs	Thiazide diuretics

Abstract: Cancer is the second highest cause of death in the world after cardiovascular disease. Despite the success of chemotherapeutic agents many patients are left with the unfortunate side effects of these drugs. Cytotoxic antibiotics such as anthracyclines and the monoclonal antibody trastuzamab are both commonly used in breast cancer and may cause irreversible cardiotoxicity, though trastuzumab-induced damage can be reversed upon stopping. Various animal studies have shown that cardioprotective drugs such as ACE inhibitors, Beta-blockers and statins can reduce pathological processes such as oxidative stress as a result of chemotherapy, however their protective role in human is currently unknown.

In this study, 1229 eligible breast cancer patients who received anthracyclines and/or trastuzumab as well as the use of cardioprotective agents were retrospectively observed to see if they have therapeutical effects in reversing myocardial damage. Furthermore, a population-based record-linkage study of all breast cancer patients in Tayside, Scotland undergoing adjuvant chemotherapy was carried out to audit the incidence of cardiotoxicity in breast cancer as well as adherence to guidelines which was never been evaluated previously. Results revealed that although the majority of patients had a baseline assessment, when they were divided into anthracycline + trastuzumab or anthracycline those receiving anthracycline only had suboptimal surveillance of cardiac function. Additionally, the latter group was observed to have a higher incidence of Left ventricular systolic dysfunction (LVSD) which is thought to be due to a lower rate of surveillance.

The hypothesis of this study was that the use of cardioprotective drugs such as ACE inhibitors, Beta blockers and ARBs as well as cholesterol controlling drugs such as statins may be able to reverse the cardiac damage caused by cancer therapy drugs such as anthracyclines how ever, the results from the overall PROTECT-TAYSIDE study were in disagreement with the hypothesis and showed that the use of cardioprotective drugs did not reverse anthracycline-induced cardiac damage.

1 Chapter 1: Literature review

1.1 Introduction:

Despite vast developments in molecular biology and biochemistry, cancer and the mechanisms involved remain partially understood. Cancer is the second commonest cause of death worldwide after cardiovascular diseases, with mortality rates reported to be as high as 8.2 million in 2012[1]. Although recent advances in medical and clinical research have turned cancer from a fatal illness to a survivable chronic disease in many cases, anti-cancer treatments can have a variety of undesirable side effects and often patients show different degrees of toxicity. Adult human cardiomyocytes are highly specialised and have complex structures that support cell growth. They enter an additional step of DNA synthesis which leaves them binucleated thus making them display in determined forms such as binucleation and hypertrophy[5]. One of the most commonly known adverse effects of chemotherapy is cardiotoxicity, whereby the cardiomyocytes are damaged due to the action/nature of anti-cancer drugs that act by killing cancer cells that divide quickly and proteins that are expressed in these cells. An example of such a drug class would be Anthracyclines (Ant) which are the most widely used group of anti-neoplastic drugs in different types of cancer including breast, ovarian, lung, lymphomas and leukaemia[1]. Their use has effectively improved survival in childhood cancers by 75% and breast cancer by 70%[1]. As cardiotoxicity is a known side effect of cancer therapy, damaged cardiomyocytes lead to impaired function of the cardiac muscle which in turn ultimately may lead to heart failure.

1.1.1 Heart failure epidemiology:

Heart failure is a complex condition where the heart's ability to pump blood around the body is impaired and it is recognised as a major public health problem in the developed countries. The prevalence of heart failure can be estimated at 1-2% with an incidence rate of 5-10 people out of 1000 per year in the western world[2]. According to figures from information services division (ISD) Scotland, Mortality rate for all heart diseases was 328 per 100,000 people between 2005, and 212 per 100,000 in 2014, a reduction of 35.6% in the last ten years[61].

1.1.2 Prevalence of Heart failure:

Heart failure is most frequently seen in people over the age 50 and the prevalence continues to increase with age. In 2000, the prevalence of heart failure in the UK was reported to be 3 per 1000 compared with 9 per 1000 in 1958 according to UK national data[3]. In 2007 Mosterd and Hoes reported the prevalence of HF in the US to be 2.2% ranging from 0.7% in individuals between 45-54 years old to 8.4% in individuals over the age of 75 in a population size of 2042 randomly selected residents[2].This US-based population study also indicated that individuals with systolic dysfunction often display subclinical symptoms of recognised heart failure[2].

1.1.3 Incidence of heart failure:

In two population-based studies the incidence of heart failure was reported to have increased from 0.2/1000 person per year to 12.4/1000 person per year in individuals of 45-55 years of age, and the second study known as the Rotterdam study the incidence increased from 2.5/1000 to 44/1000 person with an age of 55-64 years, person with a mean age of >58 years[2].

1.2 Heart failure risk factors

There are several factors that increase one's risk of developing heart failure, with the most common in the western world being Ischaemic Heart Disease (IHD). Coronary artery disease is the predominant cause of heart failure and has been shown to increase the chance of developing heart failure within 7-8 years of an episode of myocardial infarction (MI) in up to 36% of patients[2]. Although hypertension has a smaller association with heart failure than myocardial infarction it contributes significantly as it is more common in the population[2]. Physical inactivity, smoking, obesity, valvular heart disease and diabetes are other contributing risk factors for heart failure.

Although to a lesser extent, there are other risk factors such as drug side effects which can lead to cardiac dysfunction[4]. Cardiotoxicity is of a particular interest which occurs as a side effect of chemotherapy, especially the anthracycline class. As cancer survival increases, chemotherapy-induced cardiotoxicity is becoming an increasing concern as it can lead to the development of heart failure. Advanced heart failure in patients with the disease characteristics above have the same prognosis as metastatic breast cancer. In this review cardiotoxicity and possible strategies to reverse or inhibit its effect are described.

1.3 Cardiotoxicity

The National Cancer Institute defined cardiotoxicity as "toxicity that affects the heart". Although a simple description, it is one of the most feared side effects of anticancer therapy and therefore a clear understanding of how cardiotoxicity occurs and how to best prevent it remains the objective of many studies in this area[5]. According to Albini *et al*[6], cardiotoxicity is said to occur when there is a reduction in left ventricular ejection fraction (LVEF) to less than 55% baseline, 5% in symptomatic and 10% reduction in asymptomatic patients

from baseline. Other features associated with heart failure that may occur in patients with cardiotoxicity include arrhythmia and tachycardia or both, shortness of breath, fatigue dyspnea and lower limb oedema[6].

1.4 Epidemiology of cardiotoxicity related heart failure:

More than 50% of patients exposed to anthracyclines show some evidence of cardiac dysfunction and of this population, 5% develop congestive heart failure (CHF). These effects can develop up to 20 years following the last dose of chemotherapy. Anthracycline-induced cardiotoxicity can be a fatal side effect of chemotherapy with a mortality rates reported to be as high as 60%, 2 years after the last exposure to the drug[1]. A retrospective analysis by Swain *et al*[7] who looked at 630 patients diagnosed with congestive heart failure, randomised into placebo and doxorubicin group, stated that doxorubicin caused cardiotoxicity in 7% of patients manifesting as congestive heart failure, when they were given a cumulative dose of doxorubicin >550mg/m^2. Their analysis which included patients with either breast carcinoma or small cell lung carcinoma and indicated that an estimated cumulative 26% of patients would experience doxorubicin-related heart failure[7]. Retrospective analyses from clinical trials suggest that the incidence of heart failure due to doxorubicin is seen to be 1.7% at a cumulative dose of 300mg/m^2, 4.7% at 400mg/m^2 and 48% at 650mg/m^2[8]. Although the study acknowledged that epirubicin is less cardiotoxic, there was no data to show the cumulative dose of this drug and its relationship with incidence of heart failure.

This clearly shows that anthracyclines cause toxicity of the heart in a dose dependant fashion. A study by Bowles *et al*[9] looked at 12500 breast cancer patients and found the incidence of heart failure increased as the follow up time

increased, giving a statistical value of 1.2% cumulative incidence after a year since the last dose of chemotherapy compared with 4.3% in year 5[9].

1.5 Detection of cardiotoxicity:

As anti-neoplastic agents such as anthracyclines cause cardiovascular side effects it is important to detect pre-existing heart problems prior to treatment, as well as obtaining baseline measurements and monitoring the activity of the heart throughout the duration of treatment to observe for any cardiotoxic effects[10]. One of the first changes that are often looked for is reduction in LVEF and the two gold standard imaging techniques used to detect such changes are echocardiography and Multigated Radionuclide Angiography (MUGA) scanning[10].

1.5.1 Conventional Echocardiography

Echocardiography is a facility that is widely available in most district general hospitals where tissue Doppler imaging recordings are used in order to measure parameters such as ejection velocity, LV deformation and deformation rate which are closely linked to myocardial function[11]. Although the use of cardiac imaging is first line to detect cardiotoxicity, echocardiography and Doppler are limited by technique-related variability which can alter the threshold of defining cardiotoxicity. Furthermore, when LVEF is detected, it is often a late feature of cardiotoxicity with only 58% recovering systolic function thereafter[12]. Another drawback of echocardiography is that the observations are inaccurate in around 20% of cases such as in patients with high BMI.

1.5.2 MUGA scans

Multigated Radionuclide Angiography (MUGA) is a non-invasive technique and less widely available facility whereby the radionuclide ^{99M}Tc binds to erythrocytes facilitated by stannous chloride, which causes the dilution of ^{99M}Tc and it enables the cardiac blood pool to be captured and visualised with a γ-

camera[13]. At each stage of the cardiac cycle there are a series of heart planar images which allow highly reproducible volumes of the left ventricle and accurate measurements of LVEF. MUGA scans have high sensitivity and are very specific in assessing ejection fraction. Furthermore, no inter and intra-observer variability are involved which is a beneficial feature compared to echocardiography as these variabilities should be considered during LVEF and contractile dysfunction measurement by using techniques such as tissue velocity and strain imaging. However a limitation of MUGA scans is that its function may be disadvantaged by soft tissue attenuation artefacts which may expose patients to ionising radiation[14]. Another limitation is that different MUGA scans are configured differently from each other depending on the model and software parameters which might affect the threshold for normality.

1.5.3 The role of biomarkers

Although LVEF is used to monitor systolic function during and after chemotherapy, it is not efficient in earlier diagnosis of toxicity in the function of the left ventricle [15]. Several studies have looked at biomarkers in the diagnosis of cardiotoxicity. Cardiac biomarkers include cardiac troponin I (cTNI) and T (cTNT) which are used to detect myocardial damage. Unlike troponin C, cTNI and cTnT are sensitive and specific to the cardiomyocytes and their role has been extended to detect other clinical damage such as left ventricular hypertrophy and heart failure[16].

For example, Sawaya *et al*[15] assessed ultrasensitive troponin I in eighty-one newly diagnosed human epidermal growth factor receptor-2 positive (HER2+) breast cancer patients treated with anthracyclines followed by the monoclonal antibody trastuzumab. It was found that ultrasensitive troponin I predicted the subsequent development of cardiotoxicity and levels of ultrasensitive troponin I

were at their peak when the anthracycline treatment was completed[15]. This shows that ultrasensitive troponin I is useful in predicting cardiotoxicity and may guide in detecting cardiac complications after the completion of anthracycline therapy. The population that was looked at by Sawaya *et al*[15] had had a low incidence of symptomatic heart failure and cardiac mortality therefore the question of why ultrasensitive troponin I is not used routinely remains unanswered, as a much greater number of patients with longer follow-up is required[15].

Several studies have also explored the role of natriuretic peptides. These are cardiac neurohormones released from the atria known as *atrial natriuretic peptide (ANP),* and *brain natriuretic peptide (BNP)*. They are released predominantly from the ventricular myocytes in response to increased wall stress. Roziakova *et al*[17] who looked at patients receiving anthracyclines such as daunarubicin, reported that the concentration of BNP and elevated concentrations cardiac troponin T correlated with significant changes in systolic and diastolic parameters obtained from echocardiographic images, in thirty-two out of thirty-seven subjects and only five patients developed manifestation of cardiotoxicity. They also reported that this result may indicate a relationship between myocyte damage and myocardial impairment[17]. This signifies the close correlation between natriuretic peptides and the detection of anthracycline-induced myocardial injury. When taking this into consideration, it is clear that the limitations of biomarkers are similar to those of cardiac imaging, in that they can only detect cardiotoxicity when functional impairments arise. Thus novel biomarkers, sensitive enough to detect changes prior to cardiotoxic significant changes are clearly needed.

1.6 Prevention of cardiotoxicity

There are some risk factors that are related to chronic progressive cardiotoxicity including cumulative dose, rate of dose delivery, pre-existing heart defects and hypertension as well as mediastinal irradiation[18]. Several preventative measures to reduce the risk of cardiotoxicity have been proposed, including limiting cumulative doses of chemotherapy, varying the dose of anthracyclines and using less cardiotoxic anthracycline analogues. However, the addition of cardioprotective agents and detection of early signs of cardiotoxicity by biomarkers are more promising strategies[8]. Additionally the use of drugs such as desferoxamine which binds iron to aluminium, reduces the iron overload in patients on anthracyclines[50].

1.6.1 Epidemiology of breast cancer

Breast cancer (BC) is the second most common cancer after lung cancer in the western world and it is reported to be increasing in prevalence[19]. For example in 2006 over 200,000 women were affected by breast cancer in the USA with a death rate of 56000 individuals and although the incidence of breast cancer is increasing an consistent improvement in 5 years survival rate has been observed[20, 21]. This is in part due to new screening programs which have allowed for increased detection and diagnosis of breast cancer[22]. Due to the earlier median (40-59 years old compared with other types of cancer where the median age is 60-70 years) age of breast cancer diagnosis compared to other cancers, there is a slightly higher probability of developing cancer before the age of 60[19].

1.6.1.1 *Prevalence of breast cancer*

Breast cancer is the single commonest cause of death among women, accounting for about a fifth of death cases in patients between 40-50 years old. Two out of every 1000 women will recently have had breast cancer diagnosis

and 15 will have had their diagnosis made before the age of 50 which equates to a prevalence of almost 2%[23]. With the advance of treatment and knowledge of cancer, treatment has become more customised based on the type of tumours including the over expression of HER2 which involves 20-30% of all breast cancers. This equates to an absolute value of 40,000 – 60,000 American patients suffering from HER2+ type of BC[20].

1.6.1.2 *Incidence of breast cancer*

Based on an article by Ferlay *et* al[24] the incidence of breast cancer was reported to be 1.67 million new cases in 2012 equating to 25% of all cancers worldwide. With 198,000 deaths in developed countries and 324,000 in less developed regions, incidence rates vary approximately fourfold between different regions of the world ranging from 27 per 100,000 in Middle Africa and Eastern Asia to 96 per 100,000 in Western Europe. As BC now has a better prognosis than ever before, mortality rates between different regions of the world are lower, especially in the developed regions where treatments are more advanced[24].

1.6.1.3 *Risk factors of breast cancer*

Despite the classic risk factors such as age, family history, age of menarche, age of menopause and radiation exposure, there are modifiable behavioural risk factors such as physical inactivity, obesity, smoking and excessive alcohol intake which creates an overlap with cardiovascular risk factors[23]. In addition to these risk factors up to 10% of breast cancer cases are due to genetic predisposition particularly in the western world[27]. The *BRCA* gene, which is expressed in the breast tissue predominantly, expresses tumour suppressing properties to help repair damaged DNA. Mutations of this gene are seen in 40-

45% somatic mutations in tumours result in failure to repair the damaged DNA[25].

1.6.1.4 *Detection of breast cancer*

There are several methods of detecting BC. Mammograms are a radiographic imaging tool whereby the image density depicts breast epithelium, and it associate it with BC. The higher the density of the mammographic images the higher the risk of BC. Boyd *et al*[26] have stated that women with increased density covering ≥75% of the breast tissue have a four to six time greater risk of BC compared to their counters who have little or no dense tissue. The literature indicates that although high density images provide accurate evaluation of the breast tissue, they fail to detect breast cancer at its early stages[26]. This suggests that there is an increased risk of BC development between screening tests.

Another method of diagnosing breast cancer is ultrasound, as mammograms can miss breast cancer in more than 50% of patients with dense breast tissue[27]. The detection of BC has been reported to increase via the use of annual ultrasound, allowing for a detection rate of 5.3 cancers per 1000 females in the first year. Berg *et a*[28] stated that using supplemental ultrasound in conjunction with MRI or mammography, was less likely to prompt unnecessary biopsy or recalling the patients[28]. Using this extra method prevents extra financial costs and saves time for both the patient and the healthcare provider.

In certain women who are at high risk of breast cancer, MRI scanning is another method that can be used along with annual mammography. MRI has the added benefit of detecting features that may have been missed by both mammography and ultrasound screening. The detailed resolution make it a useful tool to

visualise soft tissue even if a patient has dense breasts or implants as the scanner utilises magnetic field to create a visual presentation[29].

Unfortunately MRI fails to detect all types of breast cancer although it is the method of choice for detecting certain subtypes such as ductal carcinoma in-situ and lobular breast cancer are difficult to detect[29]. These disadvantages, combined with the cost of MRI means that it is not the most cost-effective way of detecting breast cancer. Additionally, a biopsy of the breast can be examined in order to determine the existence of breast carcinoma microscopically. Although the invasive nature of this investigation is a down side, biopsy is the gold standard method for detecting cancer[29].

1.6.1.5 *Treatments of breast cancer*

Although there are several ways of treating breast cancer, there are three main categories; surgical treatment and systemic treatment and radiotherapy; each section explores the available treatment options and their relevant side effects. Additionally BC treatments that are associated with cardiotoxicity are also described.

1.6.1.5.1 Surgical intervention

Mastectomy is the oldest method used in the treatment of breast cancer where one or both breasts are removed either partially or completely. Surgical removal of the breast can also be used as a prophylactic measure in patients who are at higher risk e.g. family history of BC or a previous incidence of BC with a number of lesions and or in patients suffering from multifocal disease.[30].Though the invasive nature of this procedure is a disadvantage, in that with many other operations it involves side effects such as post-surgical infections, buildup of blood and fluid in the wound (haematoma and seroma). Nevertheless

prophylactic mastectomy reduces the risk of BC occurrence and offers psychological assurance to the patients.

Lumpectomy, also known as wide local excision is used to remove a portion of the breast or a lump containing the tumour in women with intraductal breast cancer (ductal carcinoma *in situ*) and lobular carcinoma[31]. The aim of lumpectomy is to conserve the unaffected breast tissue which offers both physical (i.e. quicker recovery) and psychological satisfaction as the breast tissue does not drastically change cosmetically.

Ensuring an oncologically safe resection of breast tumours often generates unsatisfying cosmetic results and for many years oncoplastic surgery (OPS) has been used in order to overcome this challenge. This approach combines the principles of surgical oncology with plastic surgery in order to ensure optimised oncologic safety while maintaining a good cosmetic outcome. OPS offers advantages such as wider excision of the tumour while maintaining the overall shape of the breast, although it can be more complicated than mastectomy, reduced waiting time and the elimination for a complex reconstructive surgery[32].

Adjuvant radiotherapy is also used following chemotherapy or surgery, to decrease the risk of local recurrence whereby Ionising radiation is used to kill malignant cells in a localised area of the body. Unfortunately radiotherapy is also toxic to the heart and there is a higher risk of heart failure and cardiovascular mortality especially in patients with left-sided breast tumours as they receive a higher radiation dose to the heart[33].

1.6.1.5.2 Systemic intervention

As well as surgery there are a variety of systemic treatment options used in the management of breast cancer, including chemotherapy, hormonal therapy and molecular targeted therapy. Many of the chemotherapeutic agents available today work by potentiating cytotoxic effects that damage the DNA in order to stop the abnormal cell division. One down side of these agents is that they can be non-selective, meaning that they also target cells with normal DNA and normal division[34]. Some of the most commonly used classes of drugs include alkylating agents, Anthracyclines, anti-metabolites and Taxanes[34]. As this project's main interest is Anthracycline-induced cardiotoxicity, Anthracyclines and their combination with other drugs used in the treatment of breast cancer are discussed only.

Hormonal therapy

The use of hormonal therapy as an adjuvant, increases BC survival significantly in oestrogen receptor-positive patients. It is critical to continuously use adjuvant hormonal therapy after a patient finishes their chemotherapy in order to improve survival. Tamoxifen, a selective oestrogen receptor modulator (SERM) is often prescribed for up to 10 years as a preventative measure in hormone-receptor positive women[35]. Prolonged stimulation of the ductal epithelium in the breast by oestrogen contributes to the development of BC and tamoxifen inhibits the oestrogen receptor (ER) to reduce this effect[36].

Aromatase inhibitors are another class of drugs used in the treatment of breast cancer which provide novel approaches to the endocrine treatment of breast cancer. Aromatase is an enzyme of the cytochrome P-450 superfamily, is highly expressed in the placenta, the granulosa cells of ovarian follicles and to a lesser

extent in normal and adipose tissue[37]. They work by supressing plasma oestrogen levels in post-menopausal women by inhibiting or inactivating aromatase, which is the enzyme responsible for the synthesis of oestrogen from androgenic substrates[37]. Although aromatase inhibitors can be seen as challenging tamoxifen, they are the only gold standard group of drugs for post-menopausal treatment.

1.7 Breast cancer treatment and cardiotoxicity

1.7.1 Anthracyclines

Anthracyclines are highly potent cytostatic antibiotics that have been used clinically for almost half a century. They exert their effect via the inhibition of *topoisomerase II (TOP2)*, an enzyme involved in cutting the strands of the DNA helix to induce cell death[8]. As effective as they are in treating certain types of solid tumours, they are highly toxic to the heart and intercelate the DNA within the cells. Many theories have been proposed to explain their cardiotoxic mechanism but none have been proven[38]. The most accepted theory is that anthracyclines cause oxidative stress via the formation of reactive oxygen species, through different pathways. One of the most commonly known pathways is that anthracyclines enter the cell causing redox cycling leading to the generation of oxygen free radicals in the mitochondrial respiratory chains[8]. Other mechanisms that have been speculated are the accumulation of toxic metabolites and alteration in iron metabolism where *Fe-anthracycline* complexes are generated and further increase oxidative stress. As this is only at theory level, many have suggested that toxicity due to the combination of iron and some anthracylines such as Doxorubicin (DOX**)** is not a simple additive effect.

This is due to the fact anthracyclines can alter iron homeostasis through the production of oxygen free radicals[38].

1.7.2 Targeted therapies:

Patients who are HER2+ may also be given the drug Herceptin (Trastuzumab) as an adjuvant treatment as trials have shown statistically significant reduction in recurrence risks by almost 50% and by one third in overall survival[20]. However, the risk of iatrogenic cardiotoxicity increases when anthracyclines are combined with molecular targeted therapies such as Herceptin. HER2 positive patients are more likely to have lymph node involvement, higher aggression of tumour progress, decreased expression of oestrogen receptor and increased resistance to endocrine therapy[27]. This review will only focus on anthracyclines and herceptin, as there is a higher association of cardiotoxicity with these two drugs than any other anti-neoplastics. Furthermore this study will focus on the patients with breast cancer who have been treated with these two agents. Studying this group offers advantageous data as the survival rate in breast cancer is high and patients often complete the treatment regimen successfully which allows accurate calculation of the cumulative dose of therapy from start to finish. Herceptin, a recombinant humanised monoclonal antibody targets HER2 as it is up-regulated in some breast cancer patients. Its mechanisms of action include the reduction of signal transducers such as PI3 kinase and MAP kinase cascades which are activated by HER2. The reduction in receptor signalling promotes apoptosis and cycle arrest which may result from internalisation and degradation of the HER2 receptor[39]. Unfortunately though, Herceptin is been associated with cardiac dysfunction and cardiotoxicity with an incidence of between 3% and 27% in phase II/III trials[20].

Although the exact pathophysiology of Herceptin-associated cardiotoxicity remains largely unknown, it is thought to be due blockage of HER2 receptor, which under normal circumstances protects the cardiomyocytes from stress when exposed to anthracyclines[40].

Cardiomyocytes are constitutively active cells and they have a high energy demand for ATP. When Herceptin binds to HER2, its high affinity binding dampens PI3K and MAPK signalling, meaning the mitochondria become more prone to the generation of Reactive Oxygen Species (ROS), as it cannot maintain the need for ATP production. Unlike anthracyclines, Herceptin-induced toxicity is reversed when it is discontinued and there is no evidence of dose-dependent cardiomyopathy[41].

1.8 Prophylactic properties of cardioprotective agents:

Studies at animal and human level, have shown evidence of cardioprotection when prophylactic cardioprotective agents are initiated early enough at the beginning of the course of chemotherapy. In this section the agents will be explored individually to shed light on their protective mechanisms and the pharmacological approaches that may be used to potentially reverse cardiotoxicity.

1.8.1 Cardioprotective Human studies and clinical trials

1.8.1.1 *ACE inhibitors*

ACE inhibitors have been shown to have beneficial effects on anthracycline-induced cardiotoxicity. However the full spectrum of how this occurs remains poorly understood[42]. Some believe that the inhibition of ACE could cause a reduction in interstitial myocardial fibrosis or even attenuate oxidative stress, leading to improved calcium handling which improves overall myocardial function, though the mechanism of this is unclear[8].

A study conducted by Cardinale *et al*[43] involved ACE inhibitors and looked at 473 high-dose chemotherapy (HDC) patients who received different cumulative doses depending on whether they had previously undergone anthracycline therapy or not, with an elevated troponin I. These were divided into placebo and enalapril groups and treatment was started one month after HDC was given. The study reported a lower incidence of adverse outcomes such as in LVEF impairment and increases in end-diastolic pressure (EDP) and end-systolic pressure (ESP), in the ACEi group compared to the control. It was concluded therefore that the group treated with ACEi had significantly lower levels of developing cardiotoxicity ($P<0.001$)[43]. One limiting factor of a human based study with a high number of participants is that many of the cancer patients have different chemotherapeutic regimens thus their response to different cardioprotective agents may be altered.

1.8.1.2 *β-blockers*

There is growing evidence that inhibitors of β-adrenergic signalling mitigate cardiotoxicity associated with anthracyclines, though the exact mechanism remains unexplained[8]. Studies have shown that carvedilol prevents anthracycline-induced cardiomyopathy and the development of free radicals[44]. For example Kalay *et al*[44] studied a group of patients with a malignancy that required anthracyclines and randomised them into carvedilol prior to chemotherapy for 6 months as the patients were receiving chemotherapy, or placebo and evaluated their cardiac function via echocardiography. The control group had a significant reduction in their ejection fraction (EF) of 68.9 vs 52.3 ($P<0.001$), whereas the group that received carvedilol had an ejection fraction close to their initial baseline value[44]. One drawback is that the study only

included a follow up period of six months during which time it is unlikely for many patients develop a worsening heart function[44].

Another limiting factor is the number of subjects they used in this study as they only looked at 25 participants in each group. A larger population over a longer period of time would produce a more realistic perspective.

In a randomised controlled study by Kaya *et al*[45] which looked at the cardioprotective effects of nebivolol, an increase in LV end-diastolic and end systolic diameter was observed in the placebo group compared with unchanged diameter in the nebivolol group. The placebo group also had developed a lower LVEF of 57.5% compared to 63.8%. In the nebivolol group this was statistically significant, as a P value 0.001 was achieved. Although the methodology was similar to the carvedilol study, this trial administrated nebivolol prophylacticly prior to the initiation of anthracyclines[45]. The size of the study group was also small as there were only 27 participants who were assigned to nebivelol and 18 for placebo. This again raises questions regarding the power of the results/study.

1.8.1.3 *Statins*

For years it has been speculated that statins could be used as a cardioprotective agents in cancer patients to reduce anthracycline-induced cardiomyopathy[8]. A retrospective study looked at 628 female breast cancer patients with a mean age of 51 years who were receiving anthracyclines. The primary outcome of incident HF and hospitalisation was compared between those receiving statin therapy with those who did not. The findings showed that those who received statins had a significantly reduced hazard ratio of 0.3 and a p value of 0.03 which suggests a lower rate of incident HF[46].

Although these results are promising, medication records and stage/dosage of chemotherapy data were not fully obtained, meaning other potentially cardioprotective agents such as ACEi and β-blockers could have influenced the results.

1.8.1.4 *Metformin*

Interestingly the biguanide Metformin has shown to have many cardioprotective properties and it is thought to reduce the generation of ROS via the activation of *5'-adenosine monophosphate-activated protein kinase (AMPK)* as it has been observed in animal models of HF[47]. Despite these promising findings, such studies are primarily on animal models and few preliminary studies have been done at human trial level. Although metformin treatment can reduce cancer-related mortality in diabetic patients, it is unclear whether the use of metformin translates into improved clinical outcomes for patients who are receiving standard cancer therapy. Thus the use of metformin does not necessarily suggest effective therapeutic efficacy in diabetic patients with an established cancer[48].

Bosco *et al*[49] emphasised the power of metformin's cardioprotective properties by looking at 24 women who were on metformin and had BC. It was found that patients on metformin had 40% reduced risk of BC compared with non-metformin users[49]. An indication of the total number of population looked at by Libby *et al* would have given a better scope of the overall rate of BC development in metformin patients. It can be seen that the question of how metformin prevents against cardiotoxicity remains unclear.

1.8.1.5 *Dexrazoxane*

Dexarazoxane is an iron chelating agent that changes the TOP2 configuration by tight binding to its ATP-binding sites and preventing anthracyclines from binding. It is an effective drug with cardioprotective properties and it is the only FDA approved drug used against anthracyclines induced cardiotoxicity. Its mechanism of action involves chelating iron thus reducing the number of metal ions that are produced by anthracyclines. It is thought that this leads to a reduction in free radicals which ultimately decreases oxidative stress[50].

Lipshultz *et al*[51] assigned two groups of children with acute lymphoblastic leukemia into either receiving doxorubicin alone or daxarazoxane followed by doxorubicin, in an attempt to demonstrate whether the free radical scavenging drug decreases cardiotoxicity associated with anthracyclines. They observed an increase in troponin T, a measure of cardiac damage in 35% of the participants who were treated with doxorubicin alone[51]. Although there were no significant echogardiographic changes between the two groups, it was concluded that dexarazoxane prevented or at least reduced doxorubicin-induced cardiotoxicity as reflected by troponin T elevation. They also observed an 83% event-free survival at 2.5 years since the last dose was given in both groups[51].

1.9 Cardioprotective animal studies

1.9.1 ACE inhibitors

In an animal study El-Aziz *et al*[52] evaluated ACE inhibitors Enalapril and Captopril for their anti-oxidative protective actions against anthracycline-iduced cardiac and hepatic toxicity, in rats who were randomly treated with one of the above intragastrically prior to a dose of the anthracycline Adriamycin[52].

Upon an increased lactic dehydrogenase (LDH) and lipid peroxidation which are thought to be markers of anthracycline-induced cardiotoxicity, both ACE inhibitors improved the serum levels of LDH as well as other hepatic enzymes such as glutamic oxaloacetic transaminase, indicating that they possess antioxidative properties that could have the potential to protect the heart[52].

These findings are consistent with other groups who demonstrated the scavenging action of captopril both *in vitro* and *in vivo* in order to reduce superoxide radicals, thus reducing the onset of myocardial dysfunction. Furthermore ACE inhibitors were found to significantly enhance glutathione-dependant anti-oxidant defences[52].

1.9.2 β-blockers

In a study by de Nigris *et al*[53], the cardioselective beta-blocker nebivolol and the non-selective beta-blocker carvedilol were used in Sprague Dawley rats between 10-12 weeks old, who were treated with either doxorubicin or daunorubicin. Their hearts were isolated and perfused via a cannula in the aortic root and remained at a constant pressure of 90mmHg. At different time-selected points the levels of oxidative stress, cardiac damage and nitrate were evaluated and the results revealed that oxidative stress pathways were improved significantly by the addition of beta-blocker. With regards to the action of the individual beta-blockers, nebivolol presented more prominent cardioprotection compared to carvedilol[53]. This could be due to the fact that nebivolol has additional vasodilating properties that are not shared with any other beta-blocker[53].

1.9.3 Statins

The anti-hypercholesterolemic drug Lovastatin is an inhibitor of 3-hydroxy-3methyl-CoA reductase, an enzyme involved in the metabolic pathway that produces cholesterol. *In vitro* studies have shown that lovastatin reduced doxorubicin-induced cardiomyocyte death and reduced DNA damage by mediating TOP2, which is thought to be due to impaired RAC1 signalling. RAC1 inhibition has been shown to attenuate doxorubicin-induced cardiotoxicity. Lovastatin was also found to blunt the elevation of troponin I after exposure to doxorubicin[8]. Studies have also shown that the statin known as fluvastatin, through its antioxidant and anti-inflammatory properties, reduced LV dysfunction in mice exposed to doxorubicin[8].

Another study by Feleszko *et al*[54] examined the cardioprotective effects of statins in the context of anthracycline use. 11-15 week old female mice were divided into three different tumour models of 3T3 sarcoma cells, Lewis lung carcinoma cells and colon cells. They found that the addition of lovastatin resulted in a significant reduction of troponin T levels in mice who were given doxorubicin. These findings are in agreement with previous studies that have shown that lovastatin reduces doxorubicin-induced cardiac injury. Another interesting finding was the fact that there was a significantly higher sensitivity to the combined treatment with both lovastatin and doxorubicin as compared with either agent acting alone leading to a more effective decrease in the viability of cardiomyocyte damage than each agent alone[54]. The introduction of lovastatin could therefore be looked at as a possible treatment approach in patients who are about to start anthracycline treatment, to minimise the level of cardiac

damage. Although these findings are promising, there is currently insufficient data to support their use in humans.

1.9.4 Metformin

A study by Ashour *et al*[55] used adult male rats and divided them into separated groups where some were injected with saline only, some with doxorubicin only and some with metformin + doxorubicin. As predicted, electron microscopy and histopathology evaluation showed that metformin significantly protected against doxorubicin induced cardiac injury by preventing doxorubicin from reducing cardiac levels of CoA-SH (also known as Coenzyme A) which is involved in the synthesis and oxidation of fatty acids and transferring them to the mitochondria. A key finding of this investigation is that cardiotoxicity in the metformin group was found to decrease as acetyl-CoA levels increased[55]. This rise in acetyl-CoA gives the impression that mitochondria in the cardiomyocytes, which are important sources of energy and ATP production, may be restored leading to a further reduction of free radicals[55].

1.10 Review Summary

Despite current advancements in cancer therapy, the biochemical and molecular changes involved in anthracycline-induced cardiac damage remain poorly understood. In the context of detecting cardiotoxicity, cardiac imaging techniques are limited in that detection of cardiotoxicity is usually late and irreversible at this stage. Therefore, more novel imaging techniques are clearly required.

In order to prevent cardiac damage, the cumulative dose of anthracyclines has to be limited which results in a reduced efficacy that may not produce the maximum oncologic therapeutic effect. It is therefore vitally important to

find novel approaches to reduce or even completely prevent chemotherapy-associated cardiotoxicity, to allow for optimal anti-cancer therapy to be given.

Although animal studies have suggested that commonly used cardioprotective drugs such as the ones named above could potentially have a role in reducing cardiotoxicity, the translation of these findings to humans remain unknown. The aim of this study is to explore the potential benefits of cardioprotective drugs including ACE inhibitors, beta-blockers, statins and metformin in patients receiving chemotherapy for breast cancer, to see if they could be initiated prior to chemotherapy to prevent or reduce chemotherapy-induced cardiotoxicity. This will be assessed using outcome endpoints such as new-onset of heart failure, worsening heart function on echocardiogram, cardiovascular mortality and hospitalisation due to heart failure, myocardial infarction and stroke. The findings of this study may have the potential to discovering novel therapeutic strategies for this adverse effect of cancer treatment.

2 Chapter 2: Methodology

2.1 Study design: The study was an observational population-based cohort study that was undertaken using data linkage of cancer registry, dispensed prescriptions and death records in Tayside, Scotland. Following Research and Development (R&D) and Caldicott Guardian Approvals, data for a total of 1282 breast cancer patients of which 1229 were eligible for analysis (see figure 1), was collected from the oncology ward at Ninewells Hospital in Tayside. CHEMOCARE (n=12,133 records), an electronic chemotherapy prescribing and patient scheduling system database was used to search for breast cancer patients who underwent chemotherapy. Once suitable patients were identified, parameters such as the start and end date and cumulative dose of anthracycline$_{(s)}$, as well as the start and end date and cumulative dose of herceptin (Trastuzumab) were manually extracted using the Wisdom database. This is a database used by the oncology and haematology departments and holds records of visit outcomes, a brief medical history of the patient, general status of the patient's wellbeing, dates of each cycle and date of death for deceased patients.

Upon the completion of data collection, all community prescriptions for ACE inhibitors, β-blockers, statins and metformin, for each individual were extracted. Demographic data (age, gender, etc.), cardiovascular comorbidities, biochemistry & haematology blood results, imaging (echocardiography and MUGA scans), as well as Scottish Morbidity Record (SMR01), and General Registry Office (GRO) death records were extracted via the Health Informatics Centre (HIC). The Health Informatics Centre is a University of Dundee research support unit within the Tayside

medical Science Centre (TASC) that holds records of all dispensed prescriptions since 1993 for all individuals (n=approximately 400,000) in Tayside which have been collected and stored by Medicines Monitoring Unit (MEMO) and subsequently at HIC.

It administers access to validated data sets that have been anonymised using protocols approved by the Research Ethics Committee in Tayside on a virtual environment known as Safe Haven.

2.2 Study Population: All patients (≥18 years of age) who were newly diagnosed with biopsy-confirmed breast cancer who underwent anthracycline-based chemotherapy or a combination of herceptin with an anthracycline in the case of HER2+ patients, at NHS Tayside between January 2003 to December 2014 were identified using chemocare. The date of the first cycle of chemotherapy and in some cases the date of the cancer diagnosis confirmation was considered the entry date of the study. Hypertension, diabetes, smoking any cardiovascular complications, coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), cardiac dysrhythmias, depression, overweight or obesity were the recorded co-morbidities. Patients on cardioprotective drugs (ACEi & β-blockers), cholesterol lowering agents (Statins) and the anti-diabetic drug Metformin were also identified using the wisdom database at or before the start date of chemotherapeutic regimen.

A variety of risk factors for cardiotoxicity was resulting from baseline characteristics which was considered to be positive for individuals less than 55 years old who had a history of hypertension, history of smoking, coronary artery disease and or low-density lipoprotein (LDL) of >100mg/dl.

2.3 Outcomes: The primary endpoints of interest were:

1) worsening left ventricular function on echocardiogram and/or

2) worsening left ventricular function that required the loop diuretic furosemide,

3) cardiovascular mortality and

4) new onset of heart failure that required hospitalisation after the initiation of anthracycline treatment, including hospital admission due to myocardial infarction and stroke. This was done using International Classification of Disease-Tenth Revision (ICD-10) diagnosis codes I09.9, I11.0, I13.0, I13.2, I25.5, I42.0, I42.5 – I42.9, I43., I50., P29.0.

2.4 Statistical Analysis:

Drug exposure to different classes of drugs (ACE, ARB, BB, CCBs, Anti-platelets, Metformin, Sulfonylureas, Insulin & TZDs Statins during the follow-up was determined from the prescribing records.

Each patient's follow-up continued until either date of death or the end of follow-up date (earliest of each latest date of availability for SMR01, NRS, Prescribing, Echo & MUGA datasets)

The Cox proportional hazard model

The Cox proportional hazard model included age, sex, gender, baseline heart function, eGFR and baseline use of ACE inhibitors, beta-blockers, statins and metformin as independent variables and the data will be right censored if there is an outcome-endpoint (heart failure, worsening heart function on echocardiogram or MUGA scans, cardiovascular mortality and cardiovascular hospitalization) or end of follow-up period.

Separate analysis was conducted for each of the outcome measures.

If the baseline use of drugs shows protective effect on outcome-endpoint the results will then be confirmed using time-updated cox-proportional hazard models.

Breast Cancer and Cardiotoxicity; A 10-Year Audit

Different national and international guidelines are available to monitor cardiac function in patients undergoing chemotherapy. They recommend patients planned for potentially cardiotoxic chemotherapy regimens to undergo cardiovascular assessments prior, during and after treatment, to identify cardiovascular complications. However despite the availability of these guidelines, it is unknown whether they are being adhered to in clinical practice. Additionally, the incidence of chemotherapy induced cardiotoxicity in the local hospital and how rigorously are patients surveyed as per these guidelines. Therefore, an audit was performed as a sub study of the main project with the aim of examining the assessment and incidence of cardiotoxicity in breast cancer chemotherapy over a 10-year period.

Methods:

Following Caldicott guardian approval, a retrospective population cohort study was conducted at Ninewells Hospital and Medical School, a large teaching hospital in Tayside, Scotland. 1283 patient notes were initially screened using Tayside CHEMOCARE database of which 54 patients had no records of chemotherapy regimens (e.g. cumulative dose, start and end date of therapy etc) leaving 1229 eligible patients to be studied for the audit (See figure1). Records such as type of anthracycline, adjuvant radiotherapy, combination

therapy (anthracycline + Herceptin), and age were captured from January 2003 to December 2014 to determine compliance with guideline-mandated monitoring of LV function and incidence of LVSD and HF during and up to 1 year post chemotherapy. A record data linkage was established through Health Informatics Centre using a bioinformatics platform known as *safe Haven* where

large amounts of anonymised data sets are stored. Data from echocardiography database (>100,000 echo data) was linked which uses a validated natural language algorithm to identify LV dysfunction (Elder DH et al. JACC 2011; 58: 2084-91; Swejkowski BR et al. Eur J Heart Fail 2012; 14: 162-7)[56, 57]. Other imaging modalities such as MUGA scans were extracted using the GE Xeleris software used by the nuclear medicine department to look at LVEF and determine which patients are at risk of LV dysfunction. These MUGA scans were filtered accordingly as many patients had multiple scans. This gave the opportunity to look at the dates between different scans which gave a general prospective of which patients were receiving subsequent scans during the course of treatment. Other records such as prescribing data for Furosemide and cardiovascular drugs and Scottish Morbidity records (SMR01) for heart failure hospitalisation were also linked.

Audit population:

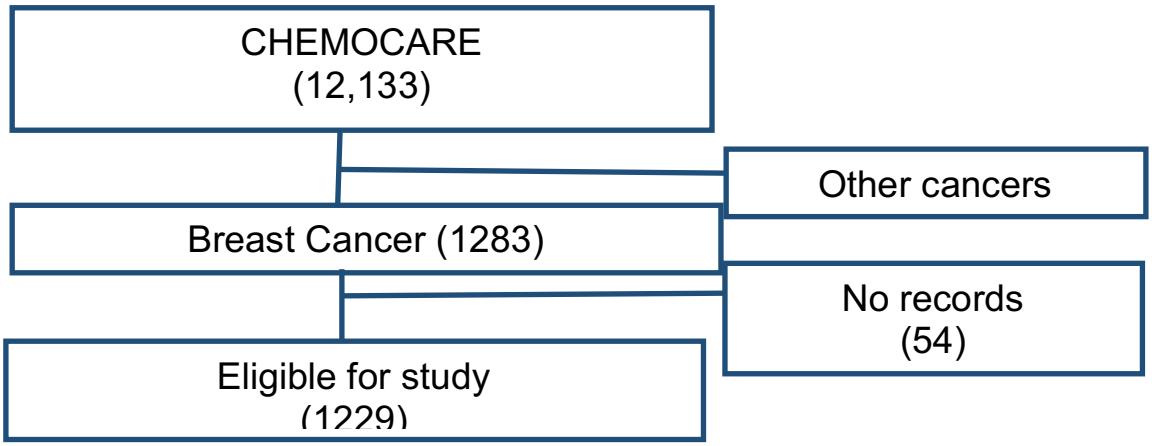

Figure 1 - Concort Figure of Population used for audit

Patient characteristics and demographics are illustrated in table 1 in the results section.

Cardiac screening

All baseline screenings for cardiac assessment were performed prior to or within 6 weeks of commencement of chemotherapy. This may seem to be a rather long period for baseline screening, however long waiting times are the main cause for such delays. These delays are sometimes inevitable as a patient may need a MUGA scan prior to the commencement of chemotherapy as safety is paramount. Of the 1229 patients only 625 (50.9%) underwent baseline cardiac assessment (mean ejection fraction 63.2% ± 7.0%) of which 238 (38.1%) had subsequent follow-up scans. All assessments were using MUGA scans and all patients had normal left ventricular ejection fraction (≥55%) with low baseline cardiovascular risk (hypertension 16.1%; diabetes 7.0%; ischaemic heart disease 3.3%) who were treated with guideline-based adjuvant chemotherapy regimens (mean cumulative doses: epirubicin 599 ±281mg/m^2; doxorubicin 559 ±143mg/m^2).

However, when patients were divided based on treatment regimes, we found 85.7% of patients treated using anthracyclines plus trastuzumab had LV function assessment at baseline and 96.9% of them had follow-up scans. This contrasted with the anthracycline only group where only 43.1% had baseline scans and a mere 12.2% were followed-up. This may be due to the belief of some healthcare providers that those who are on combination therapy may develop left ventricular systolic dysfunction quicker than those on anthracycline therapy alone.

National and International guidelines mandate the monitoring of the left ventricle before the initiation, during and after chemotherapy using imaging methods

such as echocardiography and/or MUGA scans to facilitate early detection of LVSD, as signs of cardiotoxicity begin early, during or after chemotherapy[58].

Incident LV dysfunction

Incident LV dysfunction was defined as per guidelines as follows:

- reduction of LV function by >10% AND ejection fraction of <50%
- reduction of LV function by 10%
- Worsening in qualitative LV function on echocardiogram
- Ejection fraction of <45% at anytime after baseline
- Increase in mild LV systolic dysfunction (qualitative assessment)
- Hospitalisation for heart failure AND prescription of the loop diuretic Furosemide.

The incidence of left ventricular systolic dysfunction up to a year after the completion of chemotherapy was 8.1% overall, 11.7% in the anthracycline plus trastuzumab group and 7.4% in the anthracycline only group. This is in agreement with the literature as the incident of LV dysfunction and cardiotoxicity increases significantly when patients receive a combination of anthracycline and Herceptin. The results of incidence of LV dysfunction along with other demographics and grouped treatment regimes are shown in the results section.

3 Chapter 3: Results

As mentioned in section 6.4 of methodology section, Cox proportional hazard model which included different parameters as independent variables was used to right censor the data if an outcome-endpoint such as heart failure and worsening heart function was confirmed. However, the analysis found a weak statistical signal associated with the use of cardioprotective drugs and the prevention of chemotherapy-related cardiac damage (p = NS). This in part was due to a low number of patients for the study. As the audit was done on the same study population and used the same imaging modalities, shared results were achieved and are demonstrated together below.

The demographics of patients who were studied in the audit have been tabulated below, depicting the majority of the study population to be females as breast cancer affects this gender mostly as well as different treatment regimes given and their co-morbidities. Of the 1229 patients the majority of the them received anthracycline therapy only and only 18.1% of the patients received herceptin therapy in addition to anthracycline therapy. This is in agreement with the literature as it is stated that around 20% of breast cancer patients are HER2 positive[20].

Demographics	
Total number of patients (n)	1229
Female (%)	1225 (99.7 %)
Mean age	53.84 years ± 10.7 years
Treatments received	
Patients received anthracyclines only (%)	1006 (81.9%)
Patients on combination therapy with herceptin (%)	223 (18.1%)
Herceptin therapy alone	0
Co-morbidities	
Type II Diabetes	86 (7%)
Ischaemic Heart Disease (IHD)	41 (3.3)

Table 1- Demographics of subject patients studied for a ten-year audit.

Using the statistical analysis from data collected, the cumulative dose of anthracycline in patients who received anthracycline alone showed to be 619mg/m^2 with a standard deviation of 269 and in those who received both anthracycline and herceptin received a cumulative dose of 499mg/m^2 with a standard deviation of 235.

Left ventricle assessment within 6 weeks of starting chemotherapy as well as the follow ups after that period of time are illustrated in the table 2. Results from this table show a statistically significant p value (<0.001) which show a clear evidence that the majority of patients are assessed for cardiac function within 6 weeks of treatment commencement. However this figure drops (p=0.008) which shows many of those patients on anthracycline only regimens do not receive a subsequent follow up assessment.

	A (n= 1006)	HA (n= 223)	P value
Assessment of LV function (within 6w)	625 (62.1%)		-
	434 (43.1%)	191 (85.7)	p<0.001
Follow up assessment (after 6w)	238 (38.1%)		-
	53 (12.2%)	185 (96.9%)	p=0.008

Table 2 – Percentage of patients received left ventricular assessment at baseline in both anthracycline (A) only and combination therapy (HA) patients.

	A (n=1006)		HA (n=223)		P value
Anthracycline cumulative dose (mg^2/m) (SD)	619 (269)		499 (235)		p<0.001
	No LV assesment	**LV assessment**	**No LV assessment**	**LV assessment**	
	630 (282)	606 (251)	-	-	p=0.03
	-	-	552 (117)	490 (243)	p=0.009

Table 3- Cumulative dose of anthracyline received expressed in milligrams/meter squared of surface area with the standard deviation (SD), depicting in both assessed and not assessed patients.

Following the numerical explanation of the audit in chapter 3 the incidence of LV dysfunction is represented in the table 4 below. Incidence of LV dysfunction in table 4, is shown to be lower in patients on anthracycline therapy alone compared to those on anthracycline plus adjuvant herceptin therapy.

	No Heart Failure	Heart Failure	P value
Incidence (overall population)	1114 (90.6%)	100 (8.1%)	-
anthracyclines	917 (91.2%)	74 (7.4%)	} p=0.046
Anthracycline + Herceptin	197 (88.3%)	26 (11.7%)	

Table 4 – Table showing the number of individual patients with incidence of LV dysfunction in both heart failure and no heart failure patients. Incidence is shown in different treatment groups (anthracyclines alone or anthracyline + Herceptin).

Different cardioprotective drugs show no significant protection is is provided by the drugs, meaning that there is no difference in clinical effectiveness as to whether a patient is using these drugs or not except in angiotensin receptor blockers (ARBs). An odds ratio of 2.6 portrays that patients on this ARB medication are 2.6 times more likely to develop chemotherapy-related heart failure. This is unlike what was speculated initially, as it was thought ARBs would have cardioprotective properties. This is likely to be an indication of insufficient number of study subjects with out-dated medical records. This may also mean that the effects exerted by ARBs are likely to be spurious.

	No Heart Failure	Heart Failure	P value
Anti-platelets	40 (3.6%)	2 (2%)	Insignificant
Statins	99 (8.9%)	6 (6%)	Insignificant
Beta-blockers	75 (6.7%)	6(6%)	Insignificant
ACE-inhibitors	69 (6.2%)	7 (7%)	Insignificant
ARBs	36 (3.2%)	8 (8%)	0.023 (OR = 2.6)

Table 5 – Number of individual patients on different cardioprotective drugs.

Although metformin was also one of the drugs of interest to look at, no data was received from health informatics centre at the time of analysis. This could be due an error that occurred during data linkage as many patient's records were not up to date. Although the effects of various cardio-protective drugs are insignificant, ARB effects have shown to have spurious effects. Additionally, no data on radiotherapy was collected as the main interest was to investigate the effects of chemotherapeutic agents on the heart.

Imaging Modalities

Different imaging modalities were extracted and looked at separately (see chapter (2). The two charts below show the extensive use of MUGA scans compared to echocardiography; this in part is due to the fact that MUGA scan shows a numerical measurement of ejection fraction which is a robust indicator of cardiac function in patients undergoing chemotherapy.

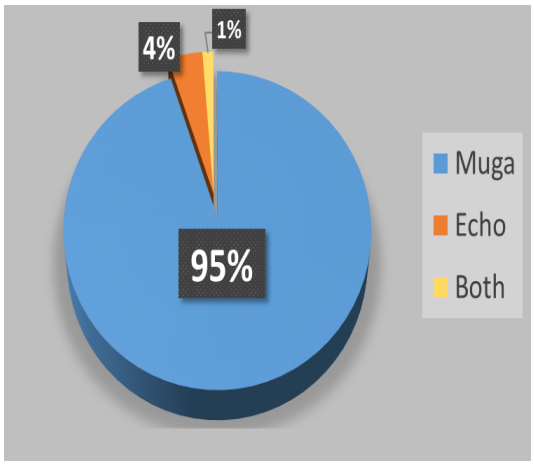

Figure 2- Modality of LV assessment at baseline level (within 6 weeks of treatment commencement).

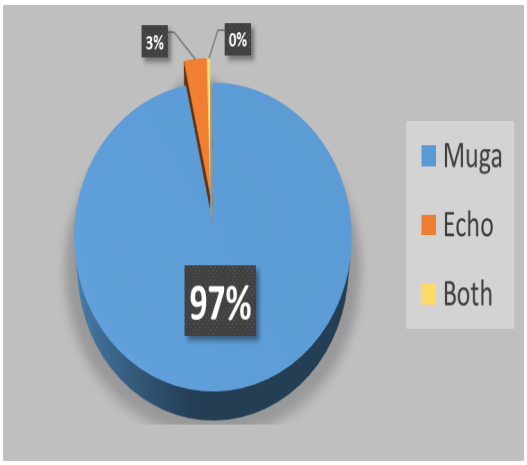

Figure 4 – Modality of LV assessment in patients with follow-up scans (>6 weeks of treatment start).

4 Chapter 4: Discussion

4.1 PROTECT-TAYSIDE study

As seen in the results section, an underpowered signal has shown that the use of cardioprotective drugs such as ACE inhibitors, ARBs, β-Blockers and statins do not protect from any chemotherapy-induced cardiotoxicity. In fact it can be seen from table 5 that contrary to what was thought, the use of ARBs causes further cardiac damage in patients receiving anthracyclines with or without adjuvant trastuzumab. Given an odds ratio of 2.6, these patients are more than twice as likely to suffer heart failure as a result of using angiotensin blockers. One possible reason could be that these patients differ from their counter parts who are receiving cancer therapy but not ARBs, in that those on ARBs may have have different fluctuations in their blood pressure due to their different action of angiotensin blockage. Interestingly this has not been reported anywhere else in the literature. The time at which this damage may occur (during or after the completion of chemotherapy) was not evaluated. Given the fact that this study was a retrospective population cohort study from a small region of Scotland, the number of available cases for the study was limited as the events have already occurred which is a limitation of retrospective studies. Having a larger number of patients for the study from a longer period of time would have been advantageous however, there were no digital records of patients prior to 2004.

Different small scale clinical trials have looked at the effects of cardioprotective drugs in the prevention of cardiac damage in patients undergoing chemotherapy regimes similar to those of this study. An example of such trial is the PRADA study by Gulati *et al*[59] conducted in 2015, which looked at 130 adult women

with early breast cancer in a 2 x 2 factorial randomised, placebo controlled trial[59]. Patients were divided into four groups and were either given candesartan cilexetil and metoprolol succinate, candesartan cilexetil and placebo, metoprolol succinate and placebo or placebo and placebo.

Different imaging modalities such as MRI and electrocardiograms were used unlike this study where MUGA and echocardiograms were used instead. However their examinations were performed at baseline and after the completion of the last cycle of anthracycline and in those on adjuvant therapy (trastuzumab or radiotherapy) at the completion of the course[59]. The aim of the PRADA trial was the same as this study with similar primary out comes such as changes in LVEF from baseline to completion of anticancer therapy determined by medical imaging. Gulati *et al*[59] reported no interaction between the use of candesartan and metoprolol and no importance was found statistically (p=0.53) or on any of their endpoints, which indicates that the drugs used did not prevent worsening of cardiac function. Interestingly effects of candesartan on changes in LVEF were not affected by adjustment for change in systolic blood pressure[59]. Although in the PROTECT-TAYSIDE study the effects of ARBs were found to be negative (OR 2.6), the overall results are in agreement with the PRADA trial.

Candesartan was found to be ineffective in causing a reduction in cardiac troponin I associated with anthracycline containing adjuvant therapy. This suggests that the blockade of angiotensin receptors may have no interface on cardiotoxic effects of anthracycline directly, but as candesartan showed a significantly less reduction in LVEF of patients who were assigned candesartan-placebo than those on placebo-placebo, it can be said that it may display a role

in the processes of remodelling of the myocardial tissue that usually occur after cardiac injury[59]. This is believed to fit with the literature both in animal and human studies.

With regards to the groups that were assigned to metoprolol, only 0.2 percentage point difference was seen between those who were on the active beta blocker and those on placebo from baseline to end of study. This equated to statistically insignificant difference with regards to reduction of anthracycline-related cardiac damage. Moreover, metoprolol showed no short term beneficial effects in the attenuation of the reduction in LV function.

The results of PROTECT-TAYSIDE study are broadly in agreement with the trial described above although the PRADA study only lasted for two years, compared with the ten-year prospective cohort analysis.

Gulati *et al*[59] believe that although inadequate statistical power was achieved, the beneficial effects of beta blockade cannot be ruled out. This projection is in agreement with this project. As the PRADA study is a randomized placebo-controlled trial, the dosage of different drugs used was adjusted by the investigators. As an example a moderately high dose of metoprolol was attained which showed a reduction in heart rate compared with the placebo group suggesting a good compliance and adequate drug efficacy[59]. Another similarity between the two studies is the limitation of not having the most up-to date follow-up records after the adjuvant therapy period, as a better understanding of the patients' condition at later stages of the diagnosis and how they were managed could have been achieved if the records were more up-to date.

Another randomized controlled study under the name of the OVERCOME trial, conducted by Bosch *et al*[60], was seeking to assess the efficacy of the ACE inhibitor enalapril and the beta-blocker carvedilol in the prevention of chemotherapy-induced LVSD in patients suffering from Haematological cancers[60]. 90 patients of which 36 were recently diagnosed with acute leukaemia and 54 with malignant haemopathies between the ages of 18 and 70 years old with normal LVEF were divided into a control and enalapril + carvedilol (taken together) groups. Both drugs were started simultaneously within at least 24 hours prior to the commencement of the first chemotherapy cycle. The primary outcome of this study was to observe changes in LVEF from baseline via echocardiography and cardiac magnetic resonance imaging 6 months after randomisation. Unlike in PROTECT-TAYSIDE study TnI and BNP were measured before and within 12 and 24 hours after each cycle. This would have been a beneficial addition to the study as it would have allowed a closer understanding of whether cardiac damage had occurred in patients.

As this is also a controlled trial, investigators had the benefit of adjusting and determining the dose administrated. As mentioned earlier, this is not a possibility with retrospective cohort studies as the study is already been done meaning only the cumulative doses of different drugs are available to analyse. The OVERCOME trial showed that concomitant treatment of enalapril and carvedilol studied may prevent LVSD in patients undergoing high doses of chemotherapy. Interestingly their use of imaging modalities (mentioned above). Although the study population of the OVERCOME trial had different malignancy compared to PROTECT-TAYSIDE's patients, one similarity between the two studies is that all included subjects had normal LVEF (>50%) at baseline[60].

Another similarity is that both the ACE inhibitor and beta-blocker used did not show any strong efficacy in patients with heart failure and preserved ejection fraction. Additionally, due to the different malignancies studied in the above trial,

more pronounced effects were seen in patients diagnosed with acute leukaemia which is contrary to the weak signal seen in PROTECT-TAYSDIE analysis. The low number of patients in OVERCOME trial was a limiting factor as the value found between the effect of intervention on LVEF and the type of disease was limited.

The results of this trial may have important clinical implications as a large number of human studies unlike animal studies have shown very small significance. However the observations made in the OVERCOME trial may prove valuable as millions of people are treated with cancer very year and a large number of them will develop cardiac damage thus the clinical relevance of combining an ACE inhibitor and a beta-blocker to prevent damage may prove valuable. To confirm this though larger studies are required.

A study by Cardinale *et al*[43] viewed consecutive cancer patients undergoing high-dose chemotherapy for different cancer conditions including breast cancer with elevated TnI who underwent anthracycline therapy. On a randomized 1:1 ratio the ACE inhibitor enalapril was given to one group while the other group was assigned for placebo over a two-year period[43]. TnI concentration was measured before and after each cycle of chemotherapy, at randomisation, prior to the administration of enalapril (in the group who received it) and up to 12 months after the first dose of enalapril. In ACE inhibitor group, enalapril was started 1 month after the completion of the last cycle of chemotherapy in order to achieve the patient's stability clinically and the ACEI treatment continued for 1 year. As with patients in the PROTECT-TAYSIDE study subjects underwent

different clinical assessments including echocardiography to assess LVEF at baseline and up to 12 months after the end of chemotherapy

on a 3 monthly period[43]. Another similarity shared was the primary endpoint which was an absolute decrease of >10 percent units at rested LVEF associated with a decrease below the normal value of 50%.

114 women were enrolled in the study of which all showed elevated TnI levels once high-dose chemotherapy had ended and of the 58 patients who were assigned to the control group, 25 individuals (43%) had a decrease in LVEF of >10% units from baseline associated with below normal limit of 50%. None of the patients in the ACE inhibitor group displayed any of the above reductions which was deemed to be a statistically significant difference ($p<0.001$). in addition to a reduced LVEF in control subjects the percentage of increased TnI was notable, indicating further cardiac damage due to inflammatory processes triggered by high doses of chemotherapy. This was evident by their Kaplan-Meier analysis where the incidence of primary endpoint was lower in the ACE inhibitor group ($p<0.001$)[43].

Unlike the analysis in PROTECT-TAYSIDE study, no CoX multivariate analysis was performed as there were no events that occurred in the ACE inhibitor group, thus the hazard ratio could not be computed and the test therefore was not applicable. good

Cardinale *et al*[43] reported the importance of using ACE inhibitors as they have shown to be effective eradicators of free radicals as they exert antioxidant effects on anthracyline-induced cardiotoxicity. The antioxidant effects are also notable in those with persistent elevated TnI especially in the first month after

the end of chemotherapy, as they had a greater chance of reduction in LVEF in the long run, if left untreated.

They concluded that an early treatment with enalapril seems to be effective in preventing cardiotoxicity and may help to improve cardiac outcome as their results showed subjects who received ACE inhibitors had a preserved left ventricular function when enalapril was used prophylactically, compared to the control group[43]. Unlike the OVERCOME study, Cadinale *et al*[43] administrated the ACE inhibitor studied prior to the start of high-dose chemotherapy and achieved a higher statistical power compared to OVERCOME. This may suggest that using an ACE inhibitor prophylactically could possibly have better cardioprotective outcomes.

All of the examples of different trials described above share the commonality of being conducted within 2 years, unlike the PROTECT-TAYSIDE study which was done over a ten-year period retrospectively. Looking at a data over a longer period of time gave the benefit of detecting any trend changes in the way breast cancer therapy is managed. As an example doxorubicin was used until 2005 and due to its more cardiotoxic nature, epirubicin was used more commonly after that period. This remark would have not been possible to see if the study was conducted over a shorter period of time.

There is a clear vision that human studies show a mixed variation of results with many showing poor statistical powers and some showing good outcomes as to whether different cardioprotective drugs reverse chemotherapy-related cardiotoxicity.

This represents a clear indication that more studies on bigger scales are required to investigate the clinical outcomes perhaps separately than investigating the drug efficacy statistically.

Looking at the literature it seems that different results and observations may be achieved if clinical effects/outcomes were compared separately from results achieved via statistical analysis. Taken the studies above together, along with other small scaled human studies, the doses of anthracycline containing chemotherapy regimens show a strong degree of association statistically, between the cumulative dose given and reduction in LVEF in the long term and not short term as ventricular-dysfunction seems to be a rare occurring event. As mentioned in section 5.4 cardiac damage and arrhythmias have been found to occur within 20 years after the treatment has been complete, thus close monitoring of patients is rather necessary to detect early signs of anthracycline-induced cardiomyopathy. Endocrine changes occur at small scales over period of time, hence the long term it takes for heart failure to occur after the last dose of chemotherapy. Other conditions such as depression and other mental issues can further speed up the occurrence of heart failure as they affect the overall cardiovascular well being of the patient.

With current medical practice several treatment strategies are used to prevent or reduce the impact of cardiac toxicity. Examples of these strategies include changing the chemotherapy schedule administered, limiting the cumulative dose of anthracycline administrated, changing the anthracycline agent to a different analogue, the use of antioxidants and iron chelators such as dexrazoxan and the stoppage of chemotherapy and restarting it again once LV recovery has been achieved. The last approach may help the LV to recover however in the majority of patients LVSD continues to deteriorate long after the

treatment has ended. This is why it would be a better practice if prevention was the main objective rather than cure. Having these treatment approaches unfortunately has the drawback of inadequate clinical success as any changes to the chemotherapeutic regimens can compromise clinical success.

As mentioned previously, the hypothesis of the PROTECT-TAYSIDE study was that agents such as ACE inhibitors, Beta-blockers and Statins thought to have a positive role in reducing or preventing anthracycline-induced cardiotoxicity. However, they were not found to have a protective effect statistically.

This may be due to the geographical location of the study not geographical location but too few numbers as the data was extracted in Tayside, Scotland which reduces the size of the study power as the data are too regional and perhaps if more data from other regions of the country was included, a higher power could have been achieved though, the limitation of this would be the amount of time and financial resources required to conduct a multi-regional study.

Like any research studies, this project also had other limitations and since this study was a retrospective population-based cohort study which unlike clinical trial studies exposure to drugs or outcomes can not be controlled and instead these have to be extracted from patient's records; this presents a problem as not all records are accurately written. This is further described below. Other challenges included limited access to CHEMOCARE and Wisdom database, as the databases were accessible from the oncology department, and the offices were available 2-3 days of the week only for data extraction which caused a longer period of data collection. The use of CHEMOCARE was limited to specific staff from the oncology ward who were not always available to help with accessing the system when required thus the only alternative was to extract

data from the Wisdom data base. However when using Wisdom many patients had missing information such as covering letters which are created on the first patient visit to the oncologist and include a list of the patient's other medications as well as a brief medical history. This often presented a challenge as even though some patients had their medications listed, the exact duration and dose of medication often were not included.

4.1 AUDIT Discussion

Data from the audit suggest that although patients receiving a combination therapy (anthracycline and trastuzumab) as adjuvant treatment are closely monitored, surveillance in those receiving anthracycline alone is low. The reason behind this discrepancy between the two groups does not seem to be clear, but may be due to the long-standing perception that damage caused by anthracycline-induced cardiotoxicity is permanent (type-1 cardiotoxicity) and that patients need only be treated when symptomatic. This could be attributed to older, less sensitive cardiac imaging modalities that could only detect cardiac dysfunction at a later stage of the disease, hence reducing the chances of recovery. However, recent work clearly documents the reversibility of this damage, and highlight the importance of early detection with more contemporary imaging techniques and prompt treatment of cardiotoxicity. The data suggests closer collaboration between oncologists and cardiologists is needed in raising awareness to this problem and improving long term overall outcomes of cancer patients. The data achieved from this audit will hopefully improve patient safety in clinical practice and more patients will undergo follow up assessments to detect cardiac complications early, as cancer therapy induced heart failure is as debilitating as the actual cancer condition.

5 Concluding remarks

Although novel anti-cancer drugs and treatment strategies have changed, breast cancer is now a survivable chronic condition. The success rate for survival has increased but this is usually at the cost of an increased risk of cardiac dysfunction due to chemotherapy-induced cardiotoxicity. Unfortunately to date the effects of cardioprotective drugs on chemotherapy-induced cardiac damage in human remains inconclusive thus larger scale trial studies with regards to the number of patients and duration of study (long-term observations) are required in order to find new treatment strategies.

Long term follow-up of patients even after the completion of chemotherapy regimens are necessary as to date, this is the best approach to detect any early signs of LV and cardiac dysfunction and prompts timely medical intervention.

6 References:

1. Kalam K, Marwick TH. Role of cardioprotective therapy for prevention of cardiotoxicity with chemotherapy: a systematic review and meta-analysis. Eur J Cancer. 2013;49(13):2900-9.

2. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. Heart. 2007;93(9):1137-46.

3. McMurray JS, S. <Heart failure - Epidemiology, aetiology and prognosis of heart failure.pdf>. Heart. 2000;83:596-602.

4. Jiang HL, G. Ogden MS, Lydia A, Bazzano. . Risk Factors for Doxorubicin-Induced Congestive Heart Failure. Arch Intern Med. 2001;161:996-1002.

5. Curigliano G, Mayer EL, Burstein HJ, Winer EP, Goldhirsch A. Cardiac toxicity from systemic cancer therapy: a comprehensive review. Prog Cardiovasc Dis. 2010;53(2):94-104.

6. Jurgens CY, Moser DK, Armola R, Carlson B, Sethares K, Riegel B, et al. Symptom clusters of heart failure. Res Nurs Health. 2009;32(5):551-60.

7. Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. Cancer. 2003;97(11):2869-79.

8. Hahn VS, Lenihan DJ, Ky B. Cancer therapy-induced cardiotoxicity: basic mechanisms and potential cardioprotective therapies. J Am Heart Assoc. 2014;3(2):e000665.

9. Bowles EJ, Wellman R, Feigelson HS, Onitilo AA, Freedman AN, Delate T, et al. Risk of heart failure in breast cancer patients after anthracycline and trastuzumab treatment: a retrospective cohort study. J Natl Cancer Inst. 2012;104(17):1293-305.

10. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Cohen V, et al. Early detection and prediction of cardiotoxicity in chemotherapy-treated patients. The American journal of cardiology. 2011;107(9):1375-80.

11. Jurcut R, Wildiers H, Ganame J, D'Hooge J, Paridaens R, Voigt JU. Detection and monitoring of cardiotoxicity-what does modern cardiology offer? Support Care Cancer. 2008;16(5):437-45.

12. Thavendiranathan P, Poulin F, Lim KD, Plana JC, Woo A, Marwick TH. Use of myocardial strain imaging by echocardiography for the early detection of cardiotoxicity in patients during and after cancer chemotherapy: a systematic review. Journal of the American College of Cardiology. 2014;63(25 Pt A):2751-68.

13. de Geus-Oei LF, Mavinkurve-Groothuis AMC, Bellersen L, Gotthardt M, Oyen WJG, Kapusta L, et al. Scintigraphic Techniques for Early Detection of Cancer Treatment-Induced Cardiotoxicity. Journal of Nuclear Medicine Technology. 2013;41(3):170-81.

14. D'Amore C, Gargiulo P, Paolillo S, Pellegrino AM, Formisano T, Mariniello A, et al. Nuclear imaging in detection and monitoring of cardiotoxicity. World journal of radiology. 2014;6(7):486-92.

15. Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Tan TC, et al. Assessment of echocardiography and biomarkers for the extended prediction of cardiotoxicity in patients treated with anthracyclines, taxanes, and trastuzumab. Circulation Cardiovascular imaging. 2012;5(5):596-603.

16. Colombo A, Sandri MT, Salvatici M, Cipolla CM, Cardinale D. Cardiac complications of chemotherapy: role of biomarkers. Curr Treat Options Cardiovasc Med. 2014;16(6):313.

17. Roziakova L, Bojtarova E, Mistrik M, Dubrava J, Gergel J, Lenkova N, et al. Serial measurements of cardiac biomarkers in patients after allogeneic hematopoietic stem cell transplantation. Journal of experimental & clinical cancer research : CR. 2012;31:13.

18. Gharib MIB, A.K. "Chemotherapy-induced cardiotoxicity current practice and pospects of prophylaxis". European Journal of Heart Failure. 2002;4:235 - 42.

19. Jemal AS, R. Xu,J. Ward,E. Cancer Statistics, 2010. Cancer Journal. 2010;60:277 - 300.

20. Engel RHK, V.G. HER2-Positive Breast Cancer.pdf. Review Article. 2007;67(9):1329 - 41.

21. Jemal AT, A. Murray,T. Thun,Michael. Cancer Statistics 2002. A Cancer Journal for Clinicians - Wiley Online Library. 2002;52:23 - 47.

22. Hanrahan EO, Gonzalez-Angulo AM, Giordano SH, Rouzier R, Broglio KR, Hortobagyi GN, et al. Overall survival and cause-specific mortality of patients with stage T1a,bN0M0 breast carcinoma. J Clin Oncol. 2007;25(31):4952-60.

23. McPherson KS, C,M. Dixon,M. <Breast cancer—epidemiology, risk factors, and genetics.pdf>. British Medical Journal. 2000;321.

24. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5):E359-86.

25. Rosen EM, Fan S, Pestell RG, Goldberg ID. BRCA1 gene in breast cancer. J Cell Physiol. 2003;196(1):19-41.

26. Boyd NFG, H. Martin,L.J. Sun,L. Stone,J. Fishell,E. Jong.R.A. Hislop,G. Chiarelli,A. Minkin,S. Yaffe,M.J. Mammographic Density and the Risk and Detection of Breast Cancer. The New England Journal of Medicine. 2007;356:227 - 36.

27. Bachawal SV, Jensen KC, Lutz AM, Gambhir SS, Tranquart F, Tian L, et al. Earlier detection of breast cancer with ultrasound molecular imaging in a transgenic mouse model. Cancer Res. 2013;73(6):1689-98.

28. Berg WAZ, Z. Lehrer,D. Jong,R. Pisano,Etta. Barr,R. Velez-Bohm,M. Mahoney,M. Evans III,W.P. Larsen,L.H. Morton,M.J. Mendelson,E.B. Farria,D.M. Cormack,J.B. Marques,H.S. Adams,A, Yeh,N.M. Gabreilli,G. . Detection of Breast Cancer with addition of annual screening ultrasound or a single screening MRI to mammography in women with elevated breast cancer risk. Journal of American Medical Association 2012;307(13).

29. Arif S, Qudsia S, Urooj S, Chaudry N, Arshad A, Andleeb S. Blueprint of quartz crystal microbalance biosensor for early detection of breast cancer through salivary autoantibodies against ATP6AP1. Biosensors & bioelectronics. 2014;65C:62-70.

30. Lostumbo LC, NE. Wallace,J. . Prophylactic mastectomy for the prevention of breast cancer (Review). The Cochrane library. 2010(11).

31. Fisher BC, H. Redmond,C. Fisher,E. Margolese,R. Lumpectomy compared with lumpectomy and radiation therapy for the treatment of intraductal breast cancer. The New England Journal of Medicine. 1993;328(22):1581 - 6.

32. Franceschini GT, D. Mango,S. Fabbri,C. Accetta,C. Di Leone,A. Moschella,F. Barbarino,R. Scaldaferri,A. D'Archi,S. Carvelli,S. Update on oncoplastic breast surgery. European Review for Medical and Pharmacological Sciences 2012;16:1530 - 40.

33. Giordano SH, Kuo YF, Freeman JL, Buchholz TA, Hortobagyi GN, Goodwin JS. Risk of cardiac death after adjuvant radiotherapy for breast cancer. J Natl Cancer Inst. 2005;97(6):419-24.

34. Pegram MD, Konecny GE, O'Callaghan C, Beryt M, Pietras R, Slamon DJ. Rational Combinations of Trastuzumab With Chemotherapeutic Drugs Used in the Treatment of Breast Cancer. JNCI Journal of the National Cancer Institute. 2004;96(10):739-49.

35. Murphy CC, Bartholomew LK, Carpentier MY, Bluethmann SM, Vernon SW. Adherence to adjuvant hormonal therapy among breast cancer survivors in clinical practice: a systematic review. Breast Cancer Res Treat. 2012;134(2):459-78.

36. Osborne CZ, H. Fuqua,S.A.W. Selective Estrogen Receptor Modulators: Structure,Function and Clinical Use. Journal of Clinical Oncology. 2000;18:3172 - 86.

37. Smith IED, M. Aromatase Inhibitors in Breast Cancer. The New England Journal of Medicine. 2003;348:2431 - 42.

38. Salazar-Mendiguchia J, Gonzalez-Costello J, Roca J, Ariza-Sole A, Manito N, Cequier A. Anthracycline-mediated cardiomyopathy: basic molecular knowledge for the cardiologist. Archivos de cardiologia de Mexico. 2014;84(3):218-23.

39. Nahta R, Esteva FJ. Herceptin: mechanisms of action and resistance. Cancer Lett. 2006;232(2):123-38.

40. Keefe DL. Trastuzumab-associated cardiotoxicity. Cancer. 2002;95(7):1592-600.

41. Zeglinski ML, A. Jassal,D.S. Singal,P.K. Trastuzumab-induced cardiac dysfunction A ‘dual-hit’. Experimental Clinical Cardiology. 2011;16(3):70-4.

42. Hiona A, Lee AS, Nagendran J, Xie X, Connolly AJ, Robbins RC, et al. Pretreatment with angiotensin-converting enzyme inhibitor improves doxorubicin-induced cardiomyopathy via preservation of mitochondrial function. The Journal of thoracic and cardiovascular surgery. 2011;142(2):396-403 e3.

43. Cardinale D, Colombo A, Sandri MT, Lamantia G, Colombo N, Civelli M, et al. Prevention of high-dose chemotherapy-induced cardiotoxicity in high-risk patients by angiotensin-converting enzyme inhibition. Circulation. 2006;114(23):2474-81.

44. Kalay N, Basar E, Ozdogru I, Er O, Cetinkaya Y, Dogan A, et al. Protective effects of carvedilol against anthracycline-induced cardiomyopathy. Journal of the American College of Cardiology. 2006;48(11):2258-62.

45. Kaya MG, Ozkan M, Gunebakmaz O, Akkaya H, Kaya EG, Akpek M, et al. Protective effects of nebivolol against anthracycline-induced cardiomyopathy: a randomized control study. International journal of cardiology. 2013;167(5):2306-10.

46. Seicean S, Seicean A, Plana JC, Budd GT, Marwick TH. Effect of statin therapy on the risk for incident heart failure in patients with breast cancer receiving anthracycline chemotherapy: an observational clinical cohort study. J Am Coll Cardiol. 2012;60(23):2384-90.

47. Asensio-López MC, Lax A, Pascual-Figal DA, Valdés M, Sánchez-Más J. Metformin protects against doxorubicin-induced cardiotoxicity: Involvement of the adiponectin cardiac system. Free Radical Biology and Medicine. 2011;51(10):1861-71.

48. Yin M, Zhou J, Gorak EJ, Quddus F. Metformin is associated with survival benefit in cancer patients with concurrent type 2 diabetes: a systematic review and meta-analysis. Oncologist. 2013;18(12):1248-55.

49. Bosco JL, Antonsen S, Sorensen HT, Pedersen L, Lash TL. Metformin and incident breast cancer among diabetic women: a population-based case-control study in Denmark. Cancer Epidemiol Biomarkers Prev. 2011;20(1):101-11.

50. Vejpongsa P, Yeh ET. Prevention of anthracycline-induced cardiotoxicity: challenges and opportunities. J Am Coll Cardiol. 2014;64(9):938-45.

51. Lipshultz SER, N. Dalton,V.M. Levy,D.E. Silvermen,L.B. Lipsitz,S.R. Colan,S.D. Asselin,B,L. Barr,R.D. Clavell,L.A. Hurwitz,C.A. Moghrabi,A. Samson,Y. Schorin,M.A. Gelber,R.D. Sallan,S.E. The effect of Dexrazoxane on Myocardial injury in Doxorubicin-treated children with acute lymphoblastic leukemia. The New England Journal of Medicine. 2004;351:145 - 53.

52. Abd El-Aziz MA, Othman AI, Amer M, El-Missiry MA. Potential protective role of angiotensin-converting enzyme inhibitors captopril and enalapril against adriamycin-induced acute cardiac and hepatic toxicity in rats. Journal of Applied Toxicology. 2001;21(6):469-73.

53. de Nigris F, Rienzo M, Schiano C, Fiorito C, Casamassimi A, Napoli C. Prominent cardioprotective effects of third generation beta blocker nebivolol against anthracycline-induced cardiotoxicity using the model of isolated perfused rat heart. European journal of cancer. 2008;44(3):334-40.

54. Fleszko WM, I. Balkowiec-Iskra,EZ. et al. Lovastatin Potentiates Antitumor Activity and Attenuates Cardiotoxicity of Doxorubicin in Three Tumor Models in Mice. American Association of Cancer Research. 2000;6:2044 - 52.

55. Ashour AE, Sayed-Ahmed MM, Abd-Allah AR, Korashy HM, Maayah ZH, Alkhalidi H, et al. Metformin Rescues the Myocardium from Doxorubicin-Induced Energy Starvation and Mitochondrial Damage in Rats. Oxidative Medicine and Cellular Longevity. 2012;2012:1-13.

56. Nadir MA, Wei L, Elder DH, Libianto R, Lim TK, Pauriah M, et al. Impact of renin-angiotensin system blockade therapy on outcome in aortic stenosis. J Am Coll Cardiol. 2011;58(6):570-6.

57. Szwejkowski BR, Elder DH, Shearer F, Jack D, Choy AM, Pringle SD, et al. Pulmonary hypertension predicts all-cause mortality in patients with heart failure: a retrospective cohort study. Eur J Heart Fail. 2012;14(2):162-7.

58. Curigliano G, Cardinale D, Suter T, Plataniotis G, de Azambuja E, Sandri MT, et al. Cardiovascular toxicity induced by chemotherapy, targeted agents and radiotherapy: ESMO Clinical Practice Guidelines. Ann Oncol. 2012;23 Suppl 7:vii155-66.

59. Gulati G, Heck SL, Ree AH, Hoffmann P, Schulz-Menger J, Fagerland MW, et al. Prevention of cardiac dysfunction during adjuvant breast cancer therapy (PRADA): a 2 x 2 factorial, randomized, placebo-controlled, double-blind clinical trial of candesartan and metoprolol. Eur Heart J. 2016;37(21):1671-80.

60. Bosch X, Rovira M, Sitges M, Domenech A, Ortiz-Perez JT, de Caralt TM, et al. Enalapril and carvedilol for preventing chemotherapy-induced left ventricular systolic dysfunction in patients with malignant hemopathies: the OVERCOME trial (preventiOn of left Ventricular dysfunction with Enalapril and caRvedilol in patients submitted to intensive ChemOtherapy for the treatment of Malignant hEmopathies). J Am Coll Cardiol. 2013;61(23):2355-62.

61. *<Scottish Heart Disease statistics.pdf>.* Information Services Division 2016: p. 17. URL: http://www.isdscotland.org/Health-Topics/Heart-Disease/Publications/2017-02-21/2017-02-21-Heart-Disease-Report.pdf

7 Bibliography:

Journals

[1] Hahn, V.S., D.J. Lenihan, and B. Ky, Cancer therapy-induced cardiotoxicity: basic mechanisms and potential cardioprotective therapies. J Am Heart Assoc, 2014. **3**(2): p. e000665.

[2] Minotti, G., *et al*., Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev, 2004. **56**(2): p. 185-229.

[3] Hanrahan, E.O., *et al*., Overall survival and cause-specific mortality of patients with stage T1a,bN0M0 breast carcinoma. J Clin Oncol, 2007. **25**(31): p. 4952-60.

[4] Pegram, M.D., *et al* Rational Combinations of Trastuzumab With Chemotherapeutic Drugs Used in the Treatment of Breast Cancer. Journal of National Cancer Institute, 2004. **96**(10): p. 739-49.

[5] Plana, J.C., *et al*., Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging, 2014. **15**(10): p. 1063-93.

[6] Armstrong, G.T., *et al*., Comprehensive Echocardiographic Detection of Treatment-Related Cardiac Dysfunction in Adult Survivors of Childhood Cancer: Results From the St. Jude Lifetime Cohort Study. J Am Coll Cardiol, 2015. **65**(23): p. 2511-22.

[7] Yeh, E.T. and P. Vejpongsa, Subclinical Cardiotoxicity Associated With Cancer Therapy: Early Detection and Future Directions. J Am Coll Cardiol, 2015. **65**(23): p. 2523-5.

Books

[1] Dale M, Rang H. Rang & Dale's pharmacology. 7th ed. Edinburgh [u.a.]: Elsevier, Churchill Livingstone; 2012.